Available online at www.sciencedirect.com
ScienceDirect
Postharvest Biology and Technology 45 (2007) 326–332
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

Control of blue mold of apple by combining controlled atmosphere, an antagonist mixture, and sodium bicarbonate

William S. Conway[a,*], Wojciech J. Janisiewicz[b], Britta Leverentz[a], Robert A. Saftner[a], Mary J. Camp[c]

[a] *Produce Quality and Safety Laboratory, Henry A. Wallace Beltsville Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705, United States*
[b] *Appalachian Fruit Research Station, Agricultural Research Service, U.S. Department of Agriculture, 45 Wiltshire Road, Kearneysville, WV 25430, United States*
[c] *Biometrical Consulting Service, Henry A. Wallace Beltsville Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705, United States*

Received 20 October 2006; accepted 11 March 2007

Abstract

'Golden Delicious' apples were wound-inoculated with *Penicillium expansum* and treated with various combinations of sodium bicarbonate and two antagonists (*Metschnikowia pulcherrima, Cryptococcus laurentii*), and then stored in air or controlled atmosphere (CA = 1.4 kPa O_2 and 3 kPa CO_2) for 2 or 4 months at 1 °C. The antagonists survived and their populations increased during both air and CA storage. The antagonists alone reduced blue mold but were more effective when combined. Sodium bicarbonate tended to reduce lesion size when used with these antagonist, either when they were used alone or combined. Storage under CA conditions also increased the effectiveness of both antagonist, when used alone or in combination. The only treatment that completely eliminated *P. expansum*-incited decay was the combination of the two antagonists and sodium bicarbonate on fruit stored under CA conditions. The proper combination of alternative control measures can provide commercially acceptable long-term control of fruit decay and could help reduce our dependency on fungicides.

Keywords: *Metschnikowia pulcherrima*; *Cryptococcus laurentii*; Postharvest biocontrol; Integrated control

1. Introduction

In a 2003 survey (http://www.foodnews.org/walletguide.php) on pesticide contamination of fresh produce, fruit topped the list of the consistently most contaminated foods and apples were the fourth most contaminated produce item. There is an increasing concern in the scientific community about the subtle ways that small doses of pesticides may have chronic adverse effects on people (Ragsdale and Sisler, 1994). Fungicides have been the most effective means of controlling postharvest diseases on fruit for many years. However, restrictions on the use of fungicides (Ragsdale and Sisler, 1994) due to the aforementioned concerns about their effects on human health and the continuing development of resistance in postharvest pathogens to the commonly used fungicides such as benzimidazole and dicarboximide (Lennox and Spotts, 2003), makes it of paramount importance to find alternatives to the use of fungicides to reduce losses from postharvest decays. Alternative control methods alone do not have as wide a spectrum of activity under various conditions as fungicides and most of them cannot achieve the effectiveness of fungicides even under optimal conditions. Therefore, a combination of promising alternatives must be used to develop a control strategy suitable for commercial application.

Biological control is an alternative to chemical control that shows effectiveness in controlling postharvest diseases (Janisiewicz et al., 2001; Janisiewicz and Jeffers, 1997; Korsten et al., 1994; Usall et al., 2001; Wilson and Wisniewski, 1989; Zhou et al., 2001). Gray mold and blue mold decay of apples and pears, caused by *Botrytis cinerea* and *Penicillium expansum,* respectively, have been controlled by bacterial and yeast antagonists (Chand-Goyal and Spotts, 1996; Janisiewicz, 1994; Janisiewicz and Marchi, 1992; Roberts, 1990). Postharvest diseases of stone, citrus, and subtropical fruit have also been

* Corresponding author at: USDA, ARS, B-002, Rm 117, 10300 Baltimore Avenue, Beltsville, MD 20705-2350, United States. Tel.: +1 301 504 6980; fax: +1 301 504 5107.
E-mail address: Conwayw@ba.ars.usda.gov (W.S. Conway).

0925-5214/$ – see front matter
doi:10.1016/j.postharvbio.2007.03.005

effectively reduced by various biocontrol agents (Janisiewicz and Korsten, 2002). Biocontrol agents are being commercially used to control postharvest decay of various fruit and vegetables. For example, Bio-Save® (JET Harvest Solutions, Orlando, FL) is being used on pome fruit, citrus fruit, cherries, seed potatoes, and sweet potatoes.

Controlled atmosphere (CA) storage has been found to be effective in delaying the onset of storage disorders in apples (Smock, 1979). Softening, bitter pit, and internal breakdown have been significantly reduced by CA storage. The effect of CA on growth and development of various decay causing fungi, including *P. expansum*, is variable and temperature related. The development of decay caused by *P. expansum* is much more effectively inhibited during cold storage in CA than in air (Nilsson et al., 1956) and the effectiveness of CA storage is increased as the temperature is reduced (Yackel et al., 1971). The growth of *P. expansum* was retarded on apples stored at 4 °C under CA conditions of 3 kPa O_2 and 5 kPa CO_2 but only slightly retarded when held in an atmosphere of 3 kPa O_2 and 0 kPa CO_2 (Borecka, 1976). In a later study, apples inoculated with *P. expansum* and stored at 0 °C under CA conditions of 3 kPa O_2 and 2 kPa CO_2 or 1 kPa O_2 and 0 kPa CO_2 had significantly less decay than fruit stored in air (Sams and Conway, 1987).

Yet another alternative is sodium bicarbonate (SBC, $NaHCO_3$), a commonly used food additive generally regarded as safe (GRAS) by the United States Food and Drug Administration. This additive has been used to reduce postharvest decay, mainly on citrus fruit (Barger, 1928). Sodium bicarbonate is a very attractive alternative because it is readily available, inexpensive, and has little risk of phytotoxicity at the low concentrations (1–4%) used (Palou et al., 2001).

The major limitations of biocontrol are a lack of eradicative activity and a narrower spectrum of activity than that found with synthetic fungicides. The reduction of decay by biological control agents is generally more variable than that for fungicides since biocontrol is more affected by environmental factors (Janisiewicz and Korsten, 2002). Also, as fruit mature, higher concentrations of the biocontrol agent must be used to achieve the same level of control as on immature fruit (Janisiewicz, unpublished data). Controlled atmosphere storage significantly reduced both sporulation and mycelial growth of *P. expansum* and may also reduce the activity of pectolytic enzymes (Yackel et al., 1971). However, once the fruit are removed from CA, there is no longer a protective effect, other than firmness retention which may make the fruit somewhat more resistant to decay, though not through any persistent inhibition on the pathogen (Kader, 1985). Likewise, SBC has no persistent protection if the fruit are re-infected after treatment (Smilanick et al., 1999).

A combination of various alternatives, including those described above, have been shown to be complementary to one another and may be integrated to equal the effect of synthetic chemicals in reducing decay of apple fruit. For example, an antagonist, *Metschnikowia pulcherrima*, was used in combination with heat (38 °C for 4 days) and 1-methylcyclo-propene treatments to control blue mold and bitter rot caused by *P. expansum* and *Colletotrichum acutatum*, respectively, on 'Golden Delicious' apples stored under CA conditions (Janisiewicz et al., 2003). Both of these diseases were controlled by this combination. In a more recent study on 'Golden Delicious' apples inoculated with either *P. expansum* or *C. acutatum* and then treated with various combinations of heat (38 °C for 4 days), 2% SBC, and two biocontrol agents (*Cryptococcus laurentii* and *M. pulcherrima*) alone or in combination, complete prevention of *C. acutatum* decay was achieved using a combination of the heat and the two antagonists (Conway et al., 2005). The antagonists alone reduced decay caused by *P. expansum* but tended to be more effective when combined. Sodium bicarbonate increased the effectiveness of decay control by each antagonist, alone or in combination. All of the treatments that included heat virtually eliminated decay caused by this pathogen. However, use of heat is still controversial and more research will need to be done before this practice is accepted by the apple industry. The effectiveness of antagonists to control diseases caused by *Penicillium* spp. on various fruit improved when they were used with SBC (Obagwu and Korsten, 2002; Porat et al., 2003; Smilanick et al., 1999).

Our research goal is to develop a strategy that will combine several effective physical and biocontrol alternatives that will provide commercially acceptable decay control under a broad range of conditions. The objective of this research was to determine the effects of combining CA storage, individual antagonists or a mixture of antagonists, and SBC treatments on reducing postharvest decay caused by *P. expansum*.

2. Materials and methods

2.1. Fruit

'Golden Delicious' apples were harvested from a commercial orchard in Pennsylvania in the preclimacteric stage (the climacteric rise in CO_2 and ethylene production had not yet occurred) and randomized on the same day as harvest. The respiration and ethylene production rates were measured over a 14-day period at 20 °C at harvest and after storage for 2 or 4 months in air at 1 °C followed by an overnight equilibration period at 20 °C. The respiration (as evolved CO_2) and ethylene production rates of seven five-fruit replications were measured every 8 h during a 14-day period using an automated system (Izumi et al., 1996). Fruit firmness was determined on 120-fruit using a manually controlled digital penetrometer (EPT-1 with an 11.1-mm tip; Lake City Technical Products, Kelowna, BC, Canada) set in the Magness–Taylor mode as previously described (Saftner et al., 1998). The starch content of 40-fruit sets was measured at harvest and following 14 days storage in air at 20 °C using the Cornell generic starch scale of 1–8 (Blanpied and Silsby, 1992).

2.2. Pathogen

The *P. expansum* isolate (MD-8) used in this study is an aggressive pathogen and has been used in our previous studies (Conway et al., 2005; Janisiewicz et al., 2003; Janisiewicz and Jeffers, 1997; Janisiewicz and Marchi, 1992). The pathogen was grown on potato-dextrose-agar (PDA) and virulence was maintained by periodic transfers through apple fruit. The conidial suspension (1×10^4 conidia mL^{-1}) used to inoculate the fruit was prepared from a 10-day-old culture as previously described (Janisiewicz and Marchi, 1992).

2.3. Antagonists

Cryptococcus laurentii (ST4-E14) and *Metschnikowia pulcherrima* (FMB-24H-2) the two antagonists used in this study, were originally isolated from surfaces of apple fruit. The yeasts were grown in 50 mL of nutrient yeast-dextrose broth medium in 250-mL Erlenmeyer flasks at 26 °C on a gyratory shaker at 150 rpm for 24 h. Then the cells were harvested by centrifuging at $7000 \times g$ for 10 min, they were re-suspended in water, and the concentration was adjusted to 3×10^7 CFU mL^{-1} with a spectrophotometer at 420 nm.

2.4. Sodium bicarbonate

Sodium bicarbonate solutions (Arm and Hammer, Princeton, NJ) at concentrations of 0 (control) or 2% (w/v) at pH 8.3–8.6 were used. The SBC concentration used was previously tested for compatibility with the two pathogens.

2.5. Controlled atmosphere

Stainless steel chambers (208 L) were used as test chambers and the desired CA (1.4 kPa O_2, 3 kPa CO_2) was obtained initially by flushing the sealed chambers containing the fruit with a mixture of CO_2 from a cylinder and humidified 1.4 kPa O_2 in N_2 supplied from a nitrogen generator (AVIR Model NA 100H5, A/G Technology Corp., Needham, MA) in series with a 5-gallon water jar. Gas concentrations were monitored with a Model S-3A Oxygen analyzer and a Model CD-3A Carbon Dioxide analyzer, both from Ametek Applied Electrochemistry, Pittsburgh, PA. Mixtures were monitored daily for the first 2 weeks and then weekly thereafter.

2.6. Fruit inoculation and treatments

The fruit were wounded with a six-penny nail to a depth of 4 mm at the equator. Each wound was inoculated with 25 μL of *P. expansum* conidial suspensions (1×10^4 mL^{-1}) alone or in combination with one or both of the antagonists (3×10^7 CFU mL^{-1}), in either 0 or 2% SBC solution. Following inoculation, one lot of fruit was placed in air storage and the other similarly treated lot was placed in CA storage. Both lots of fruit were evaluated for decay incidence and severity after storing for 2 and 4 months at 1 °C. There were three replications of nine fruit each per treatment in a completely randomized design. Severity of decay was determined by measuring the lesion diameters at each evaluation period.

2.7. Antagonist recovery

The populations of *C. laurentii* (ST4-E14) and *M. pulcherrima* (FMB-24H-2) were determined after 2 and 4 months at 1 °C. The antagonists were recovered from four wounds per treatment and enumerated separately using a previously described procedure (Conway et al., 2000).

2.8. Statistical analysis

2.8.1. Lesion severity and incidence

The severity of decay caused by *P. expansum* was determined by measuring lesion diameter at each time of evaluation. The resulting data were analyzed as a one-factor linear model using PROC MIXED (SAS Inst.) with Treatment as the effect. The assumptions of the linear model were checked and the variance grouping technique was used to correct for variance heterogeneity. The mean comparisons were done with Sidak adjusted *p*-values so that the experiment-wise error rate was 0.05. For the lesion incidence, a χ^2-analysis of the treatments was done using STATXACT 6 (Cytel Software Corp., Cambridge, MA).

2.8.2. Antagonist recovery

The populations of the yeasts resulting from the various treatments were analyzed as a three-factor linear model using PROC MIXED (SAS Inst., Cary, NC) with Antagonist, Storage Condition, and Sodium Bicarbonate as the effects. The assumptions of the linear model were checked and variance grouping was used to correct for variance heterogeneity. The mean comparisons were done with Sidak adjusted *p*-values so that the experiment-wise error rate was 0.05.

3. Results

3.1. Fruit

The respiration and ethylene production rates of the preclimacteric fruit at harvest were 50.0 ± 1.0 and 0.70 ± 0.01 pmol kg^{-1} s^{-1}, respectively. Fourteen days after harvest the fruit entered the climacteric stage of development, as indicated by rapid increases in the respiration and ethylene production rates, which were 99.0 ± 1.0 and 0.51 ± 0.01 pmol kg^{-1} s^{-1} (data not shown). Firmness at harvest was 92.9 ± 5.7 Newton (N) and starch content, using an 8-point scale (Blanpied and Silsby, 1992), was 6.7 ± 0.1.

3.2. Effect of treatments on decay

3.2.1. Decay severity

After 2 months in air or CA storage at 1 °C, the lesion diameter on control fruit stored in air was 35.5 mm and that on the CA stored fruit was significantly less at 24.2 mm (Fig. 1). The addition of SBC to these two treatments did not significantly affect lesion diameter. The lesion diameters of the *M. pulcherrima* and treated fruit in air storage were 2.7 and 1.2 mm, respectively. The addition of SBC to *M. pulcherrima* did not improve control of the decay on fruit stored in CA, although the trend was toward less decay. A combination of the antagonists was better than *M. pulcherrima* alone but not better than *C. laurentii* alone. The best treatments, resulting in no decay, were the combinations of *C. laurentii* plus SBC stored in CA, and the two

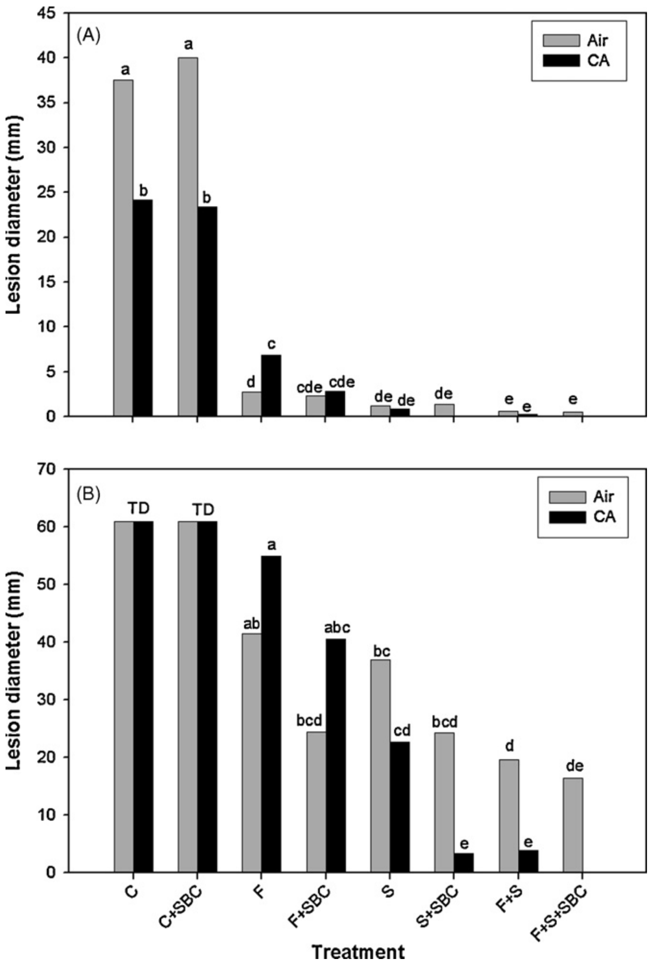

Fig. 1. Severity of blue mold on 'Golden Delicious' apples inoculated with *Penicillium expansum* alone (C) or combined with 2% sodium bicarbonate (SB), *Metschnikowia pulcherima* (F) and/or *Cryptococcus laurentii* (S), and stored at 0 °C in air or controlled atmosphere (CA; 1.4 kPa O_2 and 3 kPa CO_2) after 2 months (A) and 4 months (B). TD = fruit considered totally decayed was not included in the analysis. Bars with different letters are different at the 0.05 significance level. Bars with no letters were not included in the analysis as there was zero variance.

antagonists combined plus SBC stored in CA. After 4 months, all of the control fruit stored in air or CA were considered totally decayed (Fig. 1). The lesion diameter of *M. pulcherrima* treated fruit was 41.5 mm and adding SBC or storing in CA did not significantly reduce lesion diameter but the trend was in that direction. The lesion diameter of *C. laurentii* treated fruit was 36.9 mm and was not significantly different from the *M. pulcherrima* treated fruit. However, the lesion diameter of *C. laurentii* treated fruit plus SBC stored in CA was 3.4 mm which was the only combination that was significantly better than any combination including *M. pulcherrima* alone as well as any other combination involving *C. laurentii*. The combinations of the two antagonists and CA storage and the two antagonists plus SBC and CA storage were also as effective as the *C. laurentii* plus SBC and CA storage. Once again, no decay occurred on the fruit treated with the two antagonists plus SBC stored in CA.

Table 1
Number of apples without decay (out of 27 per treatment) at various sampling times on fruit inoculated with *Penicillium expansum* and subjected to various treatments

Treatment			Sampling time	
Antagonist[a]	SBC[b]	CA[c]	2 Months	4 Months
S + F	+	+	27	27
S	+	+	27	24
S + F	−	+	26	24
S + F	+	−	24	9
S + F	−	−	23	7
S	−	+	22	11
S	−	−	20	0
F	+	−	19	7
S	+	−	16	9
F	+	+	14	6
F	−	−	10	1
F	−	+	4	1
C	−	−	0	0
C	−	+	0	0
C	+	−	0	0
C	+	+	0	0

[a] *S*, antagonist *Cryptococcus laurentii* (ST4-E14); *F*, antagonist *Metschnikowia pulcherrima* (FMB-24H-2); *C*, control.
[b] Sodium bicarbonate (SBC) was at 2% concentration; +, treated; −, not treated.
[c] Controlled atmosphere (CA) (1.4 kPa O_2 and 3 kPa CO_2); +, treated; −, not treated (air storage).

3.2.2. Decay incidence

Decay incidence was determined by counting the number of inoculated fruit that had no lesions. The number of apples without lesions by treatment and storage time is shown in Table 1. A χ^2 analysis of the 16 treatments among fruit stored for 2 months showed that the frequency distributions were not all the same ($\chi^2 = 234.4$, p-value < .0005). Further analysis showed that the five most effective treatments were not statistically different ($\chi^2 = 8.77$, p-value = 0.077). However, the six most effective treatments were statistically different ($\chi^2 = 11.46$, p-value = 0.047). After 4 months storage, the frequency distributions were also not all the same ($\chi^2 = 234.4$, p-value = .0005). The three most effective treatments were not significantly different ($\chi^2 = 3.24$, p-value = 0.3564). However, the four most effective treatments were statistically different ($\chi^2 = 34.93$, p-value = 0.0005). After 4 months, the significantly most effective treatments were the combinations of CA, SBC, and *C. laurentii* with only 3 of the 27 fruit showing decay, a combination of *M. pulcherrima* plus *C. laurentii*, also with 3 fruit showing decay, and the best treatment was the combination of CA, SBC and *M. pulcherrima* plus *C. laurentii* in which no fruit were decayed.

3.3. Antagonist recovery

The populations of the antagonists in the wounds were from 5.94 to 6.66 log CFU/wound and from 6.07 to 6.47 log CFU/wound after 2 and 4 months of storage, respectively (Table 2). The populations of the antagonists remained stable throughout the study. Both antagonists grew equally well under air and CA storage conditions and there was no storage condition

Table 2
Recovery (log CFU/wound) of *Cryptococcus laurentii* strain ST4-E14 (S) or *Metschnikowia pulcherrima* strain FMB-24H-2 (F) from ‘Golden Delicious’ apples after 2 or 4 months storage at 1 °C in air (−) or controlled atmosphere (+) (1.4 kPa O_2 and 3 kPa CO_2) and treated with 0 (0) or 2% (2) solutions of sodium bicarbonate (SBC)

Treatment			Sampling time	
Antagonist	SBC	Storage condition	2 Months	4 months
F	0	−	5.94	6.33
F	0	+	6.13	6.19
F	2	−	6.35	6.24
F	2	+	6.16	6.07
S	0	−	6.10	6.16
S	0	+	6.43	6.25
S	2	−	6.63	6.47
S	2	+	6.66	6.42
S + F	0	−	6.51	6.22
S + F	0	+	6.60	6.32
S + F	2	−	6.50	6.23
S + F	2	+	6.49	6.47

Mean comparisons were done with Sidak adjusted *p*-values so that the experiment-wise error was 0.05. There were no statistically significant differences between the means for 2 months and for 4 months.

effect on the antagonist populations. Analysis of variance indicated that SBC had a significantly positive effect on the populations of the antagonists after 2 months in storage but not after 4 months (Table 3). Comparison of means further indicated that treatments which included SBC resulted in higher populations of both antagonists after 2 months storage (data not shown).

4. Discussion

In a previous study using the antagonists *C. laurentii* and *M. pulcherrima*, both antagonists significantly reduced decay severity and incidence caused by *P. expansum* on apple fruit (Conway et al., 2005). A combination of the two antagonists was significantly more effective than *M. pulcherrima*, but not better than *C. laurentii* alone, although the combination tended to result in smaller lesions. *M. pulcherrima* strain FMB-24H-2 was one of a number of strains found to be effective in reducing decay caused by *P. expansum* (Janisiewicz et al., 2001). This strain rapidly colonized apple wounds, grew well at 1 and 0 °C, did not grow at 37 °C, and was tolerant to diphenylamine (DPA) that is used for treatment of apples after harvest to prevent apple scald, a physiological disorder. It does not produce killer toxins and its mechanism of biocontrol is most likely competition for limiting nutrients and space at the wound site, the place of entry for the pathogen. Controlled atmosphere (CA) alone significantly reduced decay compared to regular air storage after 2 months in storage and reduced lesion size by about 40%, but after 6 months the control fruit in either air or CA storage were totally decayed. In this current study, we found that the CA conditions used had no adverse effect on the populations of antagonists *M. pulcherrima* or *C. laurentii* when compared to their populations in air storage. Both antagonists grew well and can be useful for commercial application to apples designated for CA storage. Since the effect of CA on decay development is temperature related (Yackel et al., 1971), CA should be used with the lowest temperature that would be acceptable for the commodity in question. For *P. expansum* in particular, both sporulation and mycelial growth in CA was significantly reduced as the temperature approached 2 °C (Yackel et al., 1971). CA also may reduce the activity of pectolytic enzymes but this effect varies with species (Edney, 1964). In a study with *Rhizopus stolonifer*-infected strawberries in low O_2 atmospheres at 15 °C, polygalacturonase activity from extracts of berries held at 1 kPa O_2 was one-half of that found in extracts from berries held in air storage (Wells, 1968). In a study with ‘McIntosh’ apples in CA storage, it was concluded that because CA storage tends to delay senescence and maintain fruit firmness, the improved physiological condition of the host during storage may also be responsible for retarding fungal growth (Lidster et al., 1981). CA storage, therefore, affects both the pathogen and the host. Decay development may be retarded under CA storage conditions because growth, sporulation, and enzyme activity of the pathogen is reduced and the improved physiological condition of the host enables it to resist decay more effectively.

Combining CA storage with other alternatives or a fungicide increased the effectiveness of CA storage alone in reducing decay of apples caused by *P. expansum*. Storing calcium-infiltrated fruit under CA conditions of 3 kPa O_2, 2 kPa CO_2 or 1 kPa O_2, 0 kPa CO_2 showed that combining the two treatments can result in an even greater reduction in decay and better firmness maintenance than CA or calcium treatment alone (Sams and Conway, 1987). In a more recent study, combining CA storage (2.5 kPa O_2 and 2.5 kPa CO_2) and fludioxonil at various

Table 3
Analysis of variance of the populations of the antagonists (*Cryptococcus laurentii* or *Metschnikowia pulcherrima*) recovered from wounds of apples after 2 or 4 months storage at 1 °C in air or controlled atmosphere storage (1.4 kPa O_2 and 3 kPa CO_2)

Source	DF	2 Months		4 Months	
		F-value	*p*-Value	*F*-value	*p*-Value
Antagonist	2	9.72	.003	1.37	0.29
Storage atmosphere	1	0.88	.369	0.06	0.82
Sodium bicarbonate	1	5.43	.040	1.57	0.24
Antagonist × storage atmosphere	2	0.44	.652	1.91	0.19
Antagonist × sodium bicarbonate	2	3.62	.059	3.36	0.07
Storage atmosphere × sodium bicarbonate	1	2.88	.117	0.00	0.95
Antagonist × storage condition × sodium bicarbonate	2	0.38	.692	0.64	0.54

Sodium bicarbonate concentration was 0 or 2%. For each time period, the treatments were analyzed using PROC MIXED (SAS Inst.).

concentrations, significantly lowered incidence of blue mold as compared to the standard cold storage in air (Errampalli et al., 2005). This study indicated that higher concentrations of fungicide were needed in cold storage in air than in CA to achieve similar decay control (Errampalli et al., 2005).

The use of SBC to control postharvest decay has mainly focused on citrus fruit (Obagwu and Korsten, 2002; Palou et al., 2001; Porat et al., 2003; Smilanick et al., 1999). Significant control of *Penicillium italicum* resulted when fruit were treated with 2, 3, and 4% solutions of SBC at room temperature, but 1% was ineffective (Palou et al., 2001). The SBC treatment was mainly fungistatic because it did not kill the *P. italicum* spores and so was not persistent since the pathogen survived the treatment. The presence of bicarbonate residues in the wounds was thought to be the cause of the fungistatic effect. The SBC presence delayed spore germination in the treated wounds. More recently, a 2% SBC solution killed germinating *Penicillium digitatum* spores in citrus fruit wounds (Porat et al., 2003). Germinating spores are seemingly more readily killed by SBC than non-germinating spores (Marloth, 1931).

The combination of SBC with other alternative control measures or a fungicide increased the effectiveness of these measures. The control of *P. digitatum* on citrus fruit was significantly improved by combining *Pseudomonas syringae* stain ESC10 (the active ingredient in BioSave 10; Village Farms, Biosave Division, Orlando, FL) with a 3% SBC solution (Smilanick et al., 1999). *Penicillium* spp. prefer a low-pH environment for infection and have been found to reduce the pH of the tissue in decay lesions on fruit as it develops (Prusky et al., 2004). Local alkalinization of apple fruit wounds with SBC increased wound-site pH from 4.4 to 7.1 and reduced decay caused by *P. expansum*. The effectiveness of imazalil in controlling decay of citrus fruit caused by *P. digitatum* was increased by the addition of SBC and may have been related to the elevated pH resulting from SBC (Smilanick et al., 2005). When 0.3 or 1% SBC solutions were combined with FMB-24H-2 to control *P. expansum* on apple fruit, there was no significant effect in improving the efficacy of the antagonist, probably because the SBC concentration was too low (Conway et al., 2004). However, when a 2% SBC solution was added to antagonists *C. laurentii* or *M. pulcherrima*, there was a significant reduction in decay when *M. pulcherrima* was combined with SBC, but there was no significant effect when SBC was combined with *C. laurentii* although the combination tended to result in smaller lesions (Conway et al., 2005). In this current study, although SBC combined with the antagonists did not have a statistically significant effect on lesions size, the lesions did tend to be smaller when SBC was included in the treatments.

The major advantage of chemical control is that it can completely control decay, often possess eradicative activity against existing infections, and continues to protect the host from infection in storage. To provide equivalent control, a combination of physical and/or biological alternatives is necessary, since none of the alternatives possess both protective and eradicative activities. CA storage affects decay mainly by suppressing the growth of the pathogen while in storage, but once the fruit is removed from storage, the pathogen, can resume growth. SBC has fungistatic properties, and in some cases may be fungicidal, but its effect on *P. expansum* on apple fruit has been somewhat variable. Antagonists can act as a protectant since they do protect against future infections, although in at least one instance antagonists seemed to have an eradicative effect against postharvest decays of pome and citrus fruit (Mercier and Jimenez, 2004). A combination of these alternatives, then, is necessary to develop a strategy to provide complete control. The implementation of the strategy described in this research project is more complex than the application of fungicides. However, if various fungicides are no longer effective against certain pathogens or are banned, as is the case in some countries, the strategy described herein will be an effective alternative to control postharvest apple decay caused by *P. expansum*. Finally, it is also of utmost importance that prior to the commercial adoption of any new decay control strategy, it must be ascertained that there are no adverse effects on fruit quality.

Acknowledgements

We would like to acknowledge Amy Blodgett, Willard Douglas, and Michelle Orton for valuable technical assistance. Mention of trade names or commercial products in the publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

References

Barger, W.R., 1928. Sodium bicarbonate as a citrus fruit disinfectant. Calif. Citrogr. 13, 164–174.

Blanpied, G.D., Silsby, K.J., 1992. Predicting harvest date windows for apples. Cornell Cooperative Extension Inf. Bull., 221.

Borecka, H.W., 1976. Effect of BCM fungicides on the incidence of fungi rots of apple, strawberry, and raspberry fruit, and degradation of BCM residues in fruits in CA storage. Res. Inst. Pomol. Skierniewice, Poland PL-ARS_29, FG-PO-308.

Chand-Goyal, T., Spotts, R.A., 1996. Control of postharvest pear diseases using natural saprophytic yeast colonies and their combination with a low dosage of thiabendazole. Postharvest Biol. Technol. 7, 51–64.

Conway, W.S., Leverentz, B., Saftner, R.A., Janisiewicz, W.J., Sams, C.E., Leblanc, E., 2000. Survival and growth of *Listeria monocytogenes* on fresh-cut apple slices and its interaction with *Glomerella cingulata* and *Penicillium expansum*. Plant Dis. 84, 177–181.

Conway, W.S., Leverentz, B., Janisiewicz, W.J., Blodgett, A.B., Saftner, R.A., Camp, M.J., 2004. Integrating heat treatment, biocontrol and sodium bicarbonate to reduce postharvest decay of apple caused by *Colletotrichum acutatum* and *Penicillium expansum*. Postharvest Biol. Technol. 34, 11–20.

Conway, W.S., Leverentz, B., Janisiewicz, W.J., Saftner, R.A., Camp, M.J., 2005. Improving biocontrol using antagonist mixtures with heat and/or sodium bicarbonate to control postharvest decay of apple fruit. Postharvest Biol. Technol. 36, 235–244.

Edney, K.L., 1964. The effect of the composition of the storage atmosphere on the development of rotting of Cox's Orange Pippin apples and the production of pectolytic enzymes by *Gloeosporium* spp. Annu. Rev. Biol. 54, 327–334.

Errampalli, D., Northover, J., Skog, L., Brubacher, N.R., Collucci, C.A., 2005. Control of blue mold (*Penicillium expansum*) by fludioxonil in apples (cv Empire) under controlled atmosophere and cold storage conditions. Pest Manage. Sci. 61, 591–596.

Izumi, H., Watada, A.E., Douglas, W., 1996. Optimum O_2 and CO_2 atmospheres for storing broccoli florets at various temperatures. J. Am. Soc. Hortic. Sci. 121, 127–131.

Janisiewicz, W.J., 1994. Control of storage decay of apples with *Sporobolomyces roseus*. Plant Dis. 78, 466–470.

Janisiewicz, W.J., Jeffers, S.N., 1997. Efficacy of commercial formulation of two biofungicides for control of blue and gray mold of apples in cold storage. Crop Prot. 16, 629–633.

Janisiewicz, W.J., Korsten, L., 2002. Biological control of postharvest diseases of fruits. Annu. Rev. Phytopathol. 40, 411–414.

Janisiewicz, W.J., Leverentz, B., Conway, W.S., Saftner, R.A., Reed, A.N., Camp, M.J., 2003. Control of bitter rot and blue mold of apples by integrating heat and antagonist treatments on 1-MCP treated fruit stored under controlled atmosphere conditions. Postharvest Biol. Technol. 29, 129–143.

Janisiewicz, W.J., Marchi, A., 1992. Control of storage rots on various pear cultivars with a saprophytic strain of *Pseudomonas syringae*. Plant Dis. 76, 555–560.

Janisiewicz, W.J., Tworkoski, T.J., Kurtzman, C.P., 2001. Biocontrol potential of *Metschnikowia pulcherrima* strains against blue mold of apple. Phytopathology 91, 1098–1108.

Kader, A.A., 1985. Biochemical and physiological basis for effects of controlled and modified atmospheres on fruits and vegetables. Food Technol. 40, 99–104.

Korsten, L., De Villiers, E.E., Wehner, F.C., Kotze, J.M., 1994. A review of biological control of postharvest diseases of subtropical fruits. In: Champ, B.R., Highly, E., Johnson, G.L. (Eds.), ACIAR Proc. 50. Postharvest Handling of Tropical Fruits, pp. 172–185.

Lennox, C.L., Spotts, R.A., 2003. Sensitivity of populations of *Botrytis cinerea* from pear-related sources to benzimidazole and dicarboximide fungicides. Plant Dis. 87, 645–649.

Lidster, P.D., McRae, K.B., Sanford, K.A., 1981. Response of 'McIntosh' apples to low oxygen storage. J. Am. Soc. Hortic. Sci. 106, 159–162.

Marloth, R.H., 1931. The influence of hydrogen-ion concentration and of sodium bicarbonate and related substances on *Penicillium italicum* and *P. digitatum*. Phytopathology 21, 169–198.

Mercier, J., Jimenez, J.I., 2004. Control of fungal decay of apples and peaches by the biofumigant fungus *Muscodor albus*. Postharvest Biol. Technol. 31, 1–8.

Nilsson, R., Nyhlen, A., Nilsson, R., Stoy, V., Nilson, T., 1956. Carbon dioxide storage of apples and pears 1951–1954. J. R. Swed. Acad. Agric. 93, 318–347.

Obagwu, J., Korsten, L., 2002. Integrated control of citrus green and blue molds using *Bacillum subtilis* in combination with sodium bicarbonate or hot water. Postharvest Biol. Technol. 28, 187–194.

Palou, L., Smilanick, J.L., Usall, J., Vinas, I., 2001. Control of postharvest blue and green molds of oranges by hot water, sodium carbonate, and sodium bicarbonate. Plant Dis. 85, 371–376.

Porat, R., Daus, A., Weiss, B., Cohen, L., Droby, S., 2003. Effects of combining hot water, sodium bicarbonate and biocontrol on postharvest decay of citrus fruit. J. Hortic. Sci. Biotechnol. 77, 441–445.

Prusky, D., McEvoy, J.L., Saftner, R., Conway, W.S., Jones, R., 2004. Relationship between host acidification and virulence of *Penicillium* spp. on apple and citrus fruit. Phytopathology 94, 44–51.

Ragsdale, N.N., Sisler, H.D., 1994. Social and political implications of managing plant diseases with decreased availability of fungicides in the United States. Annu. Rev. Phytopathol. 32, 545–557.

Roberts, R.G., 1990. Postharvest biological control of gray mold of apple by *Cryptococcus laurentii*. Phytopathology 80, 526–530.

Saftner, R.A., Conway, W.S., Sams, C.E., 1998. Effects of postharvest calcium and fruit coating treatments on postharvest life, quality maintenance, and fruit-surface injury in 'Golden Delicious' apples. J. Am. Soc. Hortic. Sci. 123, 294–298.

Sams, C.E., Conway, W.S., 1987. Additive effects of controlled-atmosphere storage and calcium chloride on decay, firmness retention, and ethylene production in apples. Plant Dis. 71, 1003–1005.

Smilanick, J.L., Margosan, D.A., Mlikota, F., Usall, J., Michael, M.F., 1999. Control of citrus green mold by carbonate and bicarbonate salts and the influence of commercial postharvest practices on their efficacy. Plant Dis. 83, 139–145.

Smilanick, J.L., Mansour, M.F., Margosan, D.A., Mlikota Gabler, F., 2005. Influence of pH and $NaHCO_3$ on effectiveness of imazalil to inhibit germination of *Penicillium digitatum* and to control postharvest green mold on citrus fruit. Plant Dis. 89, 640–648.

Smock, R.M., 1979. Controlled atmosphere storage of fruits. Hort. Rev. 1, 301–336.

Usall, J., Teixido, N., Torres, R., de Eribe, X.O., Vinas, I., 2001. Pilot tests of *Candida sake* (CPA-1) applications to control postharvest blue mold on apple fruit. Postharvest Biol. Technol. 21, 147–156.

Wells, J.M., 1968. Growth of *Rhizopus stolonifer* in low-oxygen atmospheres. Phytopathology 58, 1598–1602.

Wilson, C.L., Wisniewski, M.E., 1989. Biological control of postharvest diseases of fruits and vegetables: an emerging technology. Annu. Rev. Phytopathol. 27, 425–441.

Yackel, W.C., Nelson, A.I., Wei, L.S., Steinberg, M.P., 1971. Effect of controlled atmosphere on growth of mold on synthetic media and fruit. Appl. Microbiol. 22, 513–516.

Zhou, T., Chu, C.L., Liu, W.T., Schaneider, K.E., 2001. Postharvest control of blue mold and gray mold on apples using isolates of *Pseudomonas syringae*. Can. J. Plant Pathol. 23, 246–252.